Title: Under pressure: when a transformed environment pushed cells to malignancy

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Abstract: Mesenchymal stem/progenitor cells (MSPCs) have recently gained attention as key elements that contribute at different stages in oncogenesis, from predisposition to disease manifestation and evolution. In this issue of Cell Stem Cell, Zambetti, Ping, Chen et al. propose a mechanism by which mutated MSPCs transform hematopoietic cells into a malignant-prone state (Zambetti et al., 2016).

Text: MSPCs represent an important component of the specialized microenvironment (niche) where normal and leukemic hematopoietic stem and progenitor cells (HSPCs) reside in the bone marrow (BM).

Although many identified driver mutations in HSPCs can initiate leukemia, alterations in HSPC niches might contribute to leukemogenesis by providing a fertile ground for the acquisition of additional mutations, the selection of mutated clones, or impaired hematopoiesis in normal cells, which provides a competitive advantage to preleukemic cells.

A recent paper from the Raaijmakers lab (Zambetti et al., 2016) presented in this issue of *Cell Stem Cell* proposes a mechanism by which the transformed niche can trigger abnormal hematopoiesis and the accumulation of leukemic-prone HSPCs. The authors approached this question in Shwachman-Diamond Syndrome (SDS), a rare congenital disorder characterized in humans by low bone mass (osteoporosis) and a tendency to progress from ineffective blood cell production (myelodysplasia) to excessive proliferation of myeloid cells (myeloproliferation) and secondary leukemia. Human SDS carries an inactivating mutation in the *Shwachman-Bodian-Diamond Syndrome* gene (*SBDS*), which encodes a ribosomal maturation protein. Like other mutations affecting ribosomal biogenesis (named

‘‘ribosomopathies’’), SDS matches the ‘‘Dameshek’s riddle,’’ which highlights the paradoxical and still incompletely understood transition from a hypo- proliferative stage to a hyper-proliferative phase in these and other disorders (De Keersmaecker et al., 2015). Also, the consequences of the Sbds mutation in each cell type and their relative contribution in SDS have remained unclear.

In a previous study, the Raaijmakers lab specifically deleted *Sbds* in HSPCs expressing the myeloid transcription factor CCAAT/enhancer binding protein alpha (Cebpa). *Sbds* deletion activated the p53 tumor suppressor and caused apoptosis in the myeloid cells, leading to a decreased number of neutrophils, but it did not cause myelodysplasia or leukemia (Zambetti et al., 2015). To study the contribution of the mutated microenvironment, Raaijmakers et al. deleted the Sbds gene in osterix+ MSPCs in another previous study. Sbds deficiency in MSPCs caused skeletal abnormalities, myelodysplasia and sporadic leukemic transformation (Raaijmakers et al., 2010). This landmark study was among the first to show that alterations in specific niche cells can be leukemogenic. However, the underlying mechanisms had remained unclear so far.

The new study (Zambetti et al., 2016) shows structural and mechanical defects in mice lacking Sbds in MSPCs (Osx^{cre} $Sbds^{f/f}$) similar to the osteoporosis observed in human SDS. Gene ex- pression profiling of MSPCs suggested impaired osteogenic differentiation, leading to reduced numbers of bone-forming cells, which might explain the skeletal abnormalities. Interestingly, HSPC number and function were not altered. However, gene expression analysis identified signatures associated with leukemic evolution of human CD34+ cells, which included mitochondrial abnormalities. In fact, HSPCs from mice lacking Sbds in MSPCs contained hyperpolarized mitochondria that caused increased reactive oxygen species (ROS) and DNA double-strand breaks, which were marked by accumulation of Ser139-phosphorylated H2AX his- tone. Therefore, the absence of Sbds in MSPCs can cause genotoxic stress and activate DNA damage response (DDR) and DNA repair pathways in HSPCs, leading to cell-cycle arrest and apoptosis. In future studies, it will be interesting to explore how HSPCs cope with such high ROS levels and whether other BM niche cells contribute to the ability to withstand the genotoxic environment.

To elucidate the MSPC-driven induction of genotoxic stress in HSPCs, the authors focused on p53, which had been previously implicated in ribosomopathies. They found increased p53 expression and activation of its downstream pathways in *Sbds*-deleted MSPCs. Importantly, both the skeletal and the mitochondrial phenotypes were partially rescued in mice simultaneously lacking *Sbds* and *p53* in MSPCs. To search for candidate p53 targets, they compared the transcriptional profiles of *Sbds*-

deficient murine MSPCs and CD271+ MSPCs from human SDS with normal controls. They found 40 genes that were differentially expressed in both murine and human *Sbds*-deficient MSPCs. To further investigate the genes responsible for the hematopoietic abnormalities, they compared the transcriptional profile of SDS patients with that of two other related human diseases: low- risk myelodysplastic syndrome (MDS)— a preleukemic disorder with concomitant high ROS, DNA, damage and apoptosis—and Diamond Blackfan anemia (DBA; a ribosomopathy with BM failure but less prone to leukemic transformation than SDS). The rationale was to find genes implicated both in SDS and MDS –but not in DBA– as candidate drivers of leukemia predisposition. The genes encoding the S100 calcium-binding protein A8/A9 –p53 targets– were likely candidates, given their high expression in Sbds-deficient MSPCs and their known role in MDS patients (Chen et al., 2013).

S100A8/A9 are secreted inflammatory molecules that activate Toll-like receptor 4 (Tlr4) and it's downstream mediator nuclear factor-kB, leading to increased expression of Tumor necrosis factor (TNF)a (Vogl et al., 2007). Through experiments involving S100A8/A9 overexpression and inhibition, Zambetti et al. confirmed MSPC-derived S100A8/A9's capacity to induce genotoxic stress in both murine and human HSPCs (Figure 1).

Finally, the potential clinical relevance of niche S100A8/A9 expression in leukemogenesis was elegantly demonstrated after discovering a significantly higher risk of leukemic evolution in MDS patients who were classified as low-risk yet presented BM niches that were characterized by high expression of S100A8/A9.

The possibility that non-hematopoietic mutations might initiate myeloproliferative disorders is not new. This concept was first suggested in newborn mice lacking the nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor alpha (IκBα) (Rupec et al., 2005). The myeloproliferative disorder observed in Ik Ba-null mice was not recapitulated by specific IkBa deletion in myeloid and fetal liver cells. The confirmation of this initiation potential came 2 years later, when deletion of retinoic acid receptor gamma (RARg) in the hematopoietic microenvironment caused a TNFa-dependent myeloproliferative disorder (Walkley et al., 2007). Raaijmakers, Scadden, and colleagues refined the microenvironmental contributions and demonstrated that, under certain conditions, MSPCs can initiate myeloid malignancies. Deletion of the RNA processing enzyme Dicer1 in MSPCs caused MDS- like disease with sporadic transformation to AML. Loss of Dicer1 in MSPCs caused reduced Sbds expression (Raajimakers et al., 2010), Interestingly, reduced ex- pression of DICER and SBDS has been noted in MSPCs from MDS patients, suggesting potential similarities with the hu- man

disease (Santamaria et al., 2012). These and other studies that cannot be discussed due to space constraints suggest potential commonalities: bone loss and transient HSPC reduction were observed in mice lacking Dicer1 or Sbds in MSPCs and in those with a RARg-deficient BM microenvironment. Moreover, S100A8/A9-activated Tlr4 can increase TNFa, and both are required for Sbds- and RARg-deficient microenvironmental. malignancies. In turn, this inflammatory environment can also damage essential HSPC niche components, therefore facilitating disease progression (Arranz et al., 2014). Overall, these studies indicate that BM inflammation (which can be caused by mutated MSPCs) facilitates myeloproliferation and increases the risk of leukemic transformation.

Whereas it is considered that HSPC mutations are generally necessary for leukemia development, these and other future studies of preleukemic stages will determine whether functional and/or genetic microenvironmental alterations facilitate the acquisition of additional hematopoietic mutations and/or confer a competitive advantage to mutated clones, thereby providing a fertile ground for genotoxicity and/or clonal selection, respectively.

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